**Cell disruption**

Use Ultra-high Pressure Homogenizer for Cell Rupture to do the disruption, stop cycling the suspension till clarified.

**His-Tag protein purification**

**Materials**

BD Talon metal affinity resin (available from Clontech)

Equilibration / Wash buffer (50 mM sodium phosphate, 300 mM NaCl, pH 7.0 - 8.0)

Colum wash buffer (50 mM sodium phosphate, 300 mM NaCl, 5 mM imidazole, pH 7.0)

Elution buffer (50 mM sodium phosphate, 300 mM NaCl, 150 mM imidazole, pH 7.0)

10 mL gravity flow column with stopper

**Note:** Increasing the pH of the equilibration/wash buffer to 8.0 will increase binding of your target protein to the resin, but may also increase non-specific binding of other proteins. We typically use pH 7.0 for all buffers

**Protein sample preparation**

1. Re-equilibrate the raw secretion supernatant in 1x equilibration/wash buffer by dialysis or concentration/dilution. Check the pH of the sample to ensure that it is between 7.0 and 8.0
2. Concentrate the raw protein sample to a volume of < 50 mL
3. Filter the protein sample through a 0.22 um vacuum filter

**Resin preparation**

1. Resuspend the resin by inverting the bottle several times (the resin comes as a 50% slurry in 20% EtOH)
2. Pipette 2-4 mL of resin slurry (1-2 mL of resin) into a 50 mL tube. The resin has a capacity of ~ 3 mg of his-tagged protein per mL resin. If you have more protein in your sample, split the resin between 2 or more tubes
3. Spin down resin at 1000 RPM for 3 minutes
4. Remove supernatant and discard
5. Resuspend resin in 10 resin bed volumes of equilibration/wash buffer
6. Spin down resin at 1000 RPM for 3 minutes and remove supernatant

7. Repeat steps 5 and 6

**Batch binding and washes**

1. Add the concentrated protein sample to the resin
2. Incubate at 4oC for 60 minutes with gentle rocking to keep the resin in suspension
3. Spin down resin and pipette off protein supernatant without disturing the resin. Save the protein supernatant in case the protein didn't bind the resin
4. Wash - Add 20 resin bed volumes of 1x equilibration/wash buffer to the resin and incubate at 4oC for 10 minutes with gentle rocking
5. Spin down resin and remove the supernatant
6. Repeat steps 5 and 5
7. Resuspend the resin in one bed volume of equilibration/wash buffer

**Column wash and elution**

These steps are typically performed at room temperature but should be done at 4oC if you have an unstable protein

1. Transfer the resuspended resin to a clean 10 mL gravity-flow column with stopper in place
2. Allow the resin to settle out of suspension
3. Drain the remaining buffer until it reaches the top of the resin bed. Do not let the resin bed dry out
4. Wash the column once with 5 bed volumes of column wash buffer. The column wash buffer has a low concentration of imidazole which helps wash off non-specifically bound proteins
5. Elute the protein with 5 bed volumes of elution buffer. Collect the eluate in 1 mL fraction

**Resin regeneration**

The resin can be washed and reused if desired

1. Wash resin with 10 bed volumes of MES buffer, pH 5.0
2. Wash resin with 10 bed volumes of ddH20
3. Wash resin with 10 bed volumes of 20% EtOH
4. Store resin at 4oC in 20% EtOH

**Protein analysis**

1. Analyze the elution fractions by SDS-PAGE to check the purity of eluted protein. Also run the raw supernatant and wash fractions to see if any protein is being lost at other steps

**Ion-exchange purification**

**Materials**

QAE Sephadex A-25 (Amersham Pharmacia Biotech) or equivalentanion-exchange gel

Binding buffer: 20 mM Tris·Cl, pH 7.5 (or other buffer as determined empirically;see Support Protocol 1)Protein sample to be purified

Wash buffer: 20 mM Tris·Cl (pH 7.5)/100 mM NaCl (or other buffer/salt solutionas determined empirically; see Support Protocol 1)

Elution buffer: 20 mM Tris·Cl (pH 7.5)/350 mM NaCl (or other buffer/saltsolution as determined empirically; see Support Protocol 1)

Regeneration buffer: 20 mM Tris·Cl (pH 7.5)/2 M NaCl (also see SupportProtocol 5)Boiling water bath (optional)

500-ml sintered-glass filter funnel, medium porosityThree 2000-ml side-arm flasksConductivity meter

Swell and equilibrate ion-exchange gel

1. Add 10 g QAE Sephadex A-25 to 1 liter binding buffer and swell 2 days at roomtemperature or 2 hr in a boiling water bath.

The volume of swollen gel will be ～70 ml.

2. Mount 500-ml sintered-glass filter funnel on 2000-ml side-arm flask and apply suction.

3. Gently swirl swollen gel to resuspend. Pour gel slurry into funnel as fast as the fluidlevel in funnel permits, allowing buffer to collect in flask. Continue until all gel hasbeen collected. Release suction after all buffer has been removed.

4. Add 200 ml binding buffer to funnel and resuspend gel using a stirring rod. Allowresuspended gel to stand for 5. Min, then apply suction to remove buffer.5. Repeat step 4 at least three times to ensure equilibration.

The gel is considered equilibrated when the pH and salt concentration of the eluant are the same as those of the binding buffer. If the procedures here are followed, there is little need to measure pH and salt concentration of the eluant because the gel is swollen and washed in binding buffer and the chance of error is very small. The worst-case scenario is that the target protein will not bind (see Troubleshooting). The repeated washings are more for the removal of fines than for adjustment of pH or salt concentration.

6. Add enough binding buffer (~100 ml) to produce an~50% (v/v) slurry and allow gel to stand in funnel until samples are prepared.

**Adsorb sample to gel**

7. Adjust pH and salt concentration of protein sample to initial optimal values.

Samples prepared by salt precipitation may require desalting prior to ion exchange. The sample can be dialyzed against the binding buffer. Also, the salt concentration of a sample can be reduced by adding distilled water until a desired salt concentration is achieved, as measured by a conductivity meter.

8. Combine gel and sample in 2000-ml beaker or wide-mouth flask.

The ~50% gel slurry is swirled and poured from the funnel; transfer may be aided with a rubber policeman, spoon, stirring rod, or wash bottle containing binding buffer.

**Elute with step gradient of increasing salt concentration**

12. Transfer the sintered-glass funnel to the top of a clean 2000-ml side-arm flask.13. Add an equal volume of wash buffer to gel in funnel and resuspend using a stirring rod. Allow resuspended gel to stand for 5 min, then apply suction to remove buffer. Save filtrate.

The wash buffer is the first step of the gradient. The filtrate will contain sample components that were weakly adsorbed to the gel (see annotation to step 10).

14. Measure absorbance of filtrate at 280 nm using wash buffer as blank.

15. Repeat steps 13 and 14 until the last filtrate shows no significant absorbance at 280nm. Pool and save the filtrates.16. Transfer the sintered-glass funnel to the top of a clean 2000-ml side-arm flask.17. Add an equal volume of elution buffer to gel in funnel and resuspend using a stirring rod. Allow resuspended gel to stand 5 min, then apply suction to remove buffer. Save filtrate.

The elution buffer is the second step of the gradient. The filtrate will contain the sample components that were more strongly adsorbed to the gel and should include the target protein.

18. Measure the absorbance of the filtrate at 280 nm using elution buffer as a blank.19. Repeat steps 17 and 18 until the last filtrate shows no significant absorbance at 280 nm. Pool and save the filtrates for assay and subsequent purification of the target protein.

**Regenerate ion-exchange medium for reuse**

20. Transfer the sintered-glass funnel to the top of a clean 2000-ml side-arm flask.21. Add an equal volume of regeneration buffer to gel in funnel and resuspend using a stirring rod. Allow resuspended gel to stand for 5 min, then apply suction to remove buffer. Repeat five times and discard filtrates.

The regeneration buffer removes strongly bound materials from the gel.

Regenerate ion-exchange medium for reuse

22. Repeat step 11 for a total of five washes to reequilibrate the gel with binding buffer.

23. Check the pH and conductivity of the last filtrate to ensure that the gel is properly equilibrated.